Final report

GENETIC DIVERSITY AND PROFILING OF ISLAND AND TRANSLOCATED POPULATIONS OF THE BANDED HARE-WALLABY, *LAGOSTROPHUS FASCIATUS*

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Summary

- The study used genetic information to investigate differences between and within populations of the banded hare-wallaby (BHW).

- This information compared genetic diversity of individuals from naturally occurring populations from Dorre and Bernier Islands. The study also generated data from two captive breeding programs at Peron (Peron Captive Breeding Colony or PCBC) and Dryandra (Return to Dryandra Field Breeding Facility or RTD).

- Genetic variation was examined at ten nuclear genes (microsatellite) from 137 individual BHWs.

- Genetically, the species is represented by two genetic populations, each occurring on two islands (separated by approximately 500m) of about 50 km^2 each in size

- Genetic analyses show that the BHW contains low levels of diversity, a pattern that is reflected in the rufous hare-wallaby found on the same islands.

- Island populations show a 'typical' pattern of reduced variability. These levels are similar to the rufous hare-wallaby (RHW, *Lagorchestes hirsutus*) however there is no comparative population for the BHW on mainland Australia.

- Both island populations show signatures of recent and past genetic bottlenecks as a result of a population crash.

- The captive breeding colony at Peron has preserved a genetic population that almost mirrors their source population from Bernier Island, and should be viewed as a success from a management outcome.

- The RTD individuals showed a similar genetic signature to Dorre Island individuals of the BHW, however the release was not successful. Dorre Island BHWs are therefore not represented 'outside' the island.

- If another introduction is being contemplated, it may be prudent to either/or ensure an insurance population of Dorre Island individuals and consider to mix individuals from both islands in order to retain representative genetic material from all available sources.

Background

The banded hare-wallaby (BHW; *Lagostrophus fasciatus fasciatus*) is a marsupial that is naturally only found on Bernier and Dorre Islands off Western Australia. A small population has recently been established on Faure Island from Bernier Is/PCBC stock and the introduction appears to have been successful. The species previously had a wide-ranging distribution on the mainland, being found in the south-west through to South Australia.

Currently there is little information available on the genetic diversity of the island populations. However, work on the mammalian diversity on islands have identified that they are genetically depauperate, and are considered to have low levels of genetic diversity.

Specific aims

This project has four key aims:

1. To develop efficient molecular profiling technology for the BHW to test levels of genetic diversity.

2. To collate and summarise the genetic profiles of the two island populations, and to identify if either of these founder populations are genetically unique.

3. To also genotype the captive-bred stock from the PCBC to identify if genetic signatures of the Bernier Island founders are identifiable.

4. Use this information to inform management of the numbers of founders from each island required for successful translocation to Dirk Hartog Island, and to provide a genetic perspective on their management, and to provide this in a timely manner (completion before the end of 2013).

1. Introduction

Translocations are an important tool in species management because they enhance gene flow among populations which helps to prevent subpopulation isolation, thereby maintaining genetic variation and avoiding inbreeding depression (Franklin 1980 and Frankel and Soulé 1981). Implicit in these management options is the assumption that gene flow will have positive effects by acting as a creative evolutionary force in maintaining genetic variation and/ or introducing favorable migrants (i.e., well-adapted individuals with high fitness; Wright 1931 and Slatkin 1987). Habitat fragmentation can restrict gene flow, which can result in the loss of genetic variation. As a result, management strategies often include translocations among populations or captive breeding and release of individuals into natural populations. Whether these programs directly consider the effects of gene flow, they act to enhance gene flow among populations (Avise 1994).

Islands have made important contributions to the conservation of threatened species. An important factor is that there are lots of them (Abbott 2000; Abbott and Burbidge 1995; Atkinson 2002; Burbidge and Manly 2002) and they also safeguard against the threats posed to mainland or continental populations (Abbott 2000). Management options have involved the marooning as insurance onto islands (Williams 1977, Moro 2003), as they are generally free of the threats found from their original habitats (Serena 1995).

A major emphasis in the conservation of mammal species on mainland sites has been the creation of protected areas (National Parks and Nature Reserves). As such, the source of breeding animals in translocations is often from the mainland to islands (Williams 1977). These have generally failed because the threatening processes (e.g. feral predators etc) remain (Burbidge and McKenzie 1989). Islands offer some buffer and protection against the threats, at least in the short term. Islands themselves can be risky where amongst other concerns (Ricciardi and Simberloff 2009), cats and foxes can invade them if they are close to the

coastline (Burbidge and Manly 2002). It is less common for translocation to occur from islands to other islands or the mainland, although Western Australia has some exceptions, such as Rothschild's rock-wallaby, *Petrogale rothschildi* (West Lewis Is from Enderby Is), *Isoodon auratus* (Hermite Is and Doole Is from Barrow Is), *Lagorchestes conspicillatus* (Hermite Is from Barrow Is), *Leggadina lakedownensis* (Serrurier Is from Thevenard Is), *Parantechinus apicalis* (Escape Is from Boullanger Is/Whitlock Is, WA).

Australia has had a poor record of mammal extinctions and declines with ~20% (19 species) now extinct. If not for the nine species only found on islands, it may be worse (Burbidge *et al.*, 1997; Burbidge, 1999). The islands of Western Australia have made a substantial contribution to the conservation of four terrestrial mammal species that once occurred over large parts of the Australian continent (*Perameles bougainville*, *Bettongia lesueur*, *Lagorchestes fasciatus* and *Pseudomys fieldi*; Burbidge *et al.*, 1997) and also to six others that have shown marked contraction in their mainland distribution, but persist on islands (*Parantechinus apicalis*, *Isoodon auratus*, *L. hirsutus*, *Macropus eugenii*, *Petrogale lateralis* and *Mesembriomys macrourus*)

Species that occur commonly offer benefits for conservation management because of the large sample sizes and well understood natural histories, however these studies may not have relevance to rare species. The BHW or mernine is only found naturally on Bernier and Dorre Islands, off Western Australia. The species formerly occurred in an arc from Shark Bay, through the south-west of Western Australia and into South Australia (Fig. 1; Richards *et al.* 2008). They were last recorded on the Australian mainland in 1906 (see Short and Turner 1992). Their fossil remains extended across the Nullarbor Plain and into south-eastern Australia in Victoria and New South Wales. Recent osteology-based phylogeny suggests that the banded hare-wallaby are an ancient macropod lineage (Prideaux and Warbuton 2010). However, the analysis did not support the placement of the mernine within Sthenurinae, but suggest it belongs to a plesiomorphic clade which branched off from other Macropodids in the early Miocene and put forward the new subfamily Lagostrophinae.

A small population has recently been established on Faure Island from Bernier/Peron Captive Breeding Centre stock and this introduction appears to have been successful. Currently there is little information available on the genetic diversity of the island populations. However there is a growing amount of information that has shown that mammals on islands tend to be genetically depauperate, and are considered to have low levels of genetic diversity (Eldridge *et al.*1999; Eldridge *et al.* 2004; Mills *et al.* 2004).

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2. Samples and Methods (Laboratory and Analyses)

Study Areas

Ear biopsies were taken from BHWs from 11 sampling locations: Dorre Island (six locations) and Bernier Island (one location, six samples collected by J. Courtney in 1998), the captive breeding colonies at Peron (PCBC), the Dryandra Woodland (Friend and Beecham 2004) and two unknown samples (see Table 1; Fig. 1). The species only occurs naturally on Dorre (53 km^2) and Bernier Islands (44 km^2), which are ~60 km off the coast of Western Australia near Shark Bay. They are arid, uninhabited and waterless, with vegetation of low heath and scrub (Richards et al. 2001).

All available samples for the BHWs (n=137) were analysed. Fifty-six RHWs were used as a comparison group, as they are also found on Dorre (n=12) and Bernier Islands (n=24), but we also had a small number from a captive mainland population (n=20). This species is now extinct on mainland Australia (Menkhorst 2001).

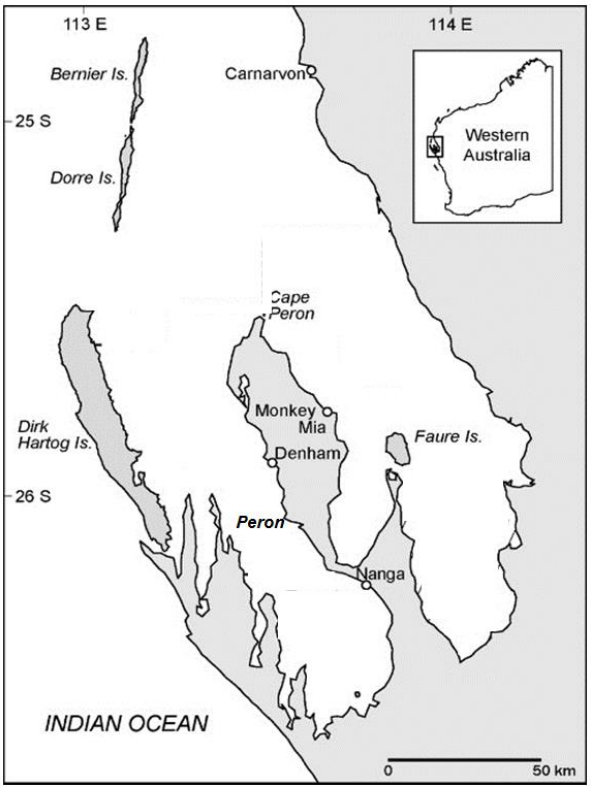


Figure 1. Sampling locations for samples of the Banded Hare-wallaby,

Molecular Methods

Nuclear (microsatellite) amplification and analysis

Ten microsatellite loci were amplified using primers derived from the tammar wallaby *Macropus eugenii* (Me14, Me17; Taylor and Cooper 1998), *Petrogale xanthopus* (Y105, Y175, Y151, Y148; Pope *et al.* 1996) and *P. assimilis* (Pa593, Pa297, Pa385, Pa55; Spencer *et al*. 1995). Briefly, PCRs were carried out in a total volume of 30 µl with ~100 ng DNA, 1X PCR buffer, 400 µM of dNTPs, 2mM $MgCl_2$, 0.2 µM of each primer & 0.825 U *Taq*. Size was determined by co-running a Genescan500 standard (Applied Biosystems, Melbourne). Fluorescently-labelled DNA fragments were separated using an ABI373*xl* capillary sequencer (Applied Biosystems) and scored manually with the aid of GENEMARKER software (v1.5, Soft Genetics). Data was checked for input errors and duplicate genotypes using the Excel Microsatellite Toolkit add-in (Park 2001). Deviations from Hardy-Weinberg equilibrium, linkage disequilibria and the presence of null alleles were tested using HW-QUICKCHECK (Kalinowski 2006), GENEPOP, (Raymond & Rousset 1995) and MICROCHECKER (van Oosterhout et al. 2004), respectively.

Population structure was inferred using STRUCTURE v 2.3 (Pritchard *et al.* 2000), based on repeated simulations from K=1 to K=10 inferred populations, using 10^6 iterations of a Markov Chain Monte Carlo (MCMC) simulation and a burn-in period of 50,000 iterations. The optimum values of *K* was determined using an ad hoc statistic (ΔK) based on the rate of change in the log probability of data between successive *K* values as described by Evanno *et al.* (2005). The level of genetic differentiation among populations was determined by estimating F_{ST} (denoted as θ, Weir & Cockerham 1984), using Fisher's exact tests for genetic differentiation from allele frequencies (Goudet *et al.* 1996) as well as R_{ST} and Rho (FSTAT 2.9.3; Goudet 1995 and GENALEX 6.3). Descriptive statistics were calculated using GenAlEx v 6.3 (Peakall and Smouse 2006) and included the number of alleles (N_A) and effective alleles per locus (N_E), as well as observed and expected heterozygosities. We estimated the genetic effective population size using linkage disequilibrium for each inferred population, implemented using the program LNDE (Waples & Do, 2008).

Detecting a change in the demographic history used a method developed by Luikart *et al.* (1998) and tested for distortion of allele frequency distributions (Luikart and Cornuet 1998) as a result of rarer alleles being more likely to be lost during a bottleneck than common alleles. This test for a genetic bottleneck is more appropriate for populations that has been reduced very recently, with less severity and the pre-bottleneck value of θ was small (see Williamson-Natesan 2005). The bottleneck results in an excess of heterozygosity under a stepwise mutation model. To detect this, we use the program BOTTLENECK 1.2 (*Piry et al.* 1999). Due to the relatively small number of loci analysed (n = 8), a Wilcoxon sign-rank test was estimated, as recommended by Piry *et al.* (1999). A mixed model of microsatellite mutation was assumed, with single step mutations assumed to account for 90% of all mutation events, and a variance among multiple steps of 12, as suggested by Piry *et al.* (1999).

Population geneticists often need to estimate individual heterozygosity (heterozygosity of individuals averaged across a panel of genetic markers). This is especially the case in recovery programs, where there is a general understanding that correlates an individual's level of heterozygosity with general fitness. The generally accepted feeling in conservation (genetics) is that more variable individuals have higher fitness. We used the program GENHET is a function written for the program R (Ihaka and Gentleman 1996). We calculate four different individual heterozygosity estimates:

- proportion of heterozygous loci (PHt) in an individual: PHt = number of heterozygous loci / number of genotyped loci.

- standardized heterozygosity based on the mean expected heterozygosity (Hs_exp, Coltman 1999): Hs_exp = PHt / mean expected heterozygosity of typed loci.

- internal relatedness (IR) (Amos 2001): IR = (2H – Σ fi) / (2N – Σ fi), where H is the number of loci that are homozygous, N is the number of loci and fi is the frequency of the i^{th} allele contained in the genotype. The maximum value (+1) is obtained when all loci are homozygous, regardless of allelic frequencies; whereas the minimum value (-1.0) can be obtained only when

all loci present only two alleles and the individual is heterozygous for all of them.

- homozygosity by locus (HL) (Aparicio *et al.* 2006): HL = Σ Eh / (Σ Eh + Σ Ej), where Eh and Ej are the expected heterozygosities of the loci that an individual bears in homozygosis (h) and in heterozygosis (j), respectively. The larger the number, the MORE homozygous the genotype is. Therefore, the smaller the number, the more heterozygous the individual.

Aparicio *et al.* (2006) performed a simulation study comparing the performances of PHt, IR and HL under different scenarios (diversity, effective population size, immigration, genetic dissimilarity, immigrants and mutation). They concluded that indices based on direct allele frequencies, like IR, may be more efficient in populations with high inbreeding (such as we expect in the BHW); whereas HL may be better in populations with migration, admixture of founders or other processes increasing genetic variability

3. Results

Genetic diversity and population genetic 'health'

A total of 137 BHWs, and 56 RHW samples were successfully scored at 10 microsatellite loci. Four loci were fixed in the BHW samples, but polymorphic in the RHW. We identified only a small number of alleles in the pooled BHW sample (27 alleles, with an average of 2.7 ± 0.62 alleles/locus; Table 1) and between 1 and 7 alleles at any single microsatellite marker and an effective number of 1.6 ± 0.3 alleles. Heterozygosity was reduced in the all of the sampled locations of BHW (average 28.2 ± 8.7%), Dorre Island containing the most genetic diversity (heterozygosity of 32%), and Bernier Island with 25% diversity.

In general, the individual measures of genetic diversity amongst sampling locations of the BHW were remarkable consistent, and differed little from the overall means (Table 1). Genetic diversity measured as both observed and expected heterozygosity was between 10 and 30%, regardless of location. Similarly, the alleles found in the locations varied little, and were found to be between 1.5 to 2.5 alleles per location, on average (Table 1). Allele frequencies (Supplementary Table 1) were used to infer that the two unknown samples originated from Bernier Island.

This contrasts with the relatively small sample from the RHW in which we identified 70 unique alleles, with a mean of 7.0 ± 0.9 alleles per locus, and expected heterozygosity of 64%. These estimates were between 50-60% higher than found in the BHW samples (Table 1). The BHW showed fixation values (*F*) that suggest the populations show random mating (Table 2).

Table 1. Measures of microsatellite variability at 10 loci for the three sampled populations of hare-wallabies. *n*, number of individuals genotypes; values given as a mean ± S.E. (standard error).

Population (sampled)	N	Number of alleles	Effective number of alleles	Expected heterozygosity	Observed heterozygosity
Dorre Island					
White Beach	20	2.2 ± 0.4	1.8 ± 0.2	0.372 ± 0.106	0.331 ± 0.094
Castle	1	1.2 ± 0.1	1.2 ± 0.1	0.200 ± 0.133	0.200 ± 0.133
Disaster Cove	1	1.3 ± 0.2	1.3 ± 0.2	0.300 ± 0.153	0.300 ± 0.153
Pinnacle	4	1.8 ± 0.2	1.4 ± 0.1	0.225 ± 0.079	0.275 ± 0.081
Quion Bluff	1	1.2 ± 0.1	1.2 ± 0.1	0.200 ± 0.133	0.200 ± 0.133
South	1	1.3 ± 0.2	1.3 ± 0.2	0.300 ± 0.153	0.300 ± 0.153
Average	26.8	2.2 ± 0.4	1.7 ± 0.2	0.323 ± 0.092	0.332 ± 0.094
Bernier Island					
Hospital Bay	12	2.0 ± 0.4	1.6 ± 0.3	0.161 ± 0.063	0.238 ± 0.088
1998 samples	7	1.8 ± 0.3	1.6 ± 0.3	0.310 ± 0.124	0.293 ± 0.102
unknown*	2	1.3 ± 0.2	1.2 ± 0.1	0.150 ± 0.076	0.150 ± 0.076
Average	19.8	2.0 ± 0.4	1.6 ± 0.3	0.258 ± 0.091	0.200 ± 0.075
Translocated/captive breeding stock					
Peron (Captive breeding colony)	82	2.2 ± 0.4	1.6 ± 0.2	0.232 ± 0.087	0.250 ± 0.087
Dryandra captive colony	6	2.1 ± 0.4	1.6 ± 0.2	0.350 ± 0.110	0.303 ± 0.090
Average	77.3	2.6 ± 0.6	1.6 ± 0.3	0.258 ±0.088	0.242 ± 0.086
Average (for BHW)	41.3	2.7 ± 0.62	1.6 ± 0.1	0.282 ± 0.050	0.251 ± 0.049
Rufous hare wallaby (*L. hirsutus*)					
Bernier	24	2.5 ± 0.3	1.6 ± 0.2	0.305 ± 0.093	0.350 ± 0.075
Dorre	12	1.7 ± 0.7	3 ± 0.	0.206 ± 0.066	0.200 ± 0.036
Mainland	20	4.6 ± 0.7	3.3 ± 0.6	0.588 ± 0.103	0.555 ± 0.306
Average (for RHW)	17.0	7.0 ± 0.9	3.2 ± 0.4	0.642 ± 0.078	0.391 ± 0.101

Table 2 No genetic bottlenecks were found in any hare-wallaby populations. Fixation index values around 0 suggest mating is random, +1 highly inbreed. *Genetic bottlenecks were only tested for pooled samples, due to the limited samples from some locations.

Population (sampled)	N	Fixation Index (*F*)	Genetic bottleneck*	Significance (*P*-value)	Mode-shift*
Dorre Island	**28**	**-0.05 ± 004**	**Yes**	**0.0156**	**Yes**
White Beach	20	-0.17 ± 0.06			
Castle	1	-1.00 ± 0.00			
Disaster Cove	1	-1.00 ± 0.00			
Pinnacle	4	0.06 ± 0.15			
Quion Bluff	1	-1.00 ± 0.00			
South	1	-1.00 ± 0.00			
Bernier Island	**21**	**0.31 ± 0.09**	**No**	**0.0781**	**Yes**
Hospital Bay	12	0.30 ± 0.10			
1998 samples	7	-0.10 ± 0.13			
unknown*	2	-0.33 ± 0.00			
Translocated/captive breeding stock					
Peron (Captive breeding colony)	73	0.08 ± 0.07	No	0.4218	Yes
Dryandra captive colony	6	-0.28 ± 0.13	No	0.5781	No
Average (BHW)					
Rufous hare wallaby (*L. hirsutus*)					
Bernier	24	-0.16 ± 0.06	No	0.9629	Yes
Dorre	12		No	0.1255	Yes
Mainland	20	0.06 ± 0.13	No	0.2734	No
Total		-0.20 ± 0.0416			

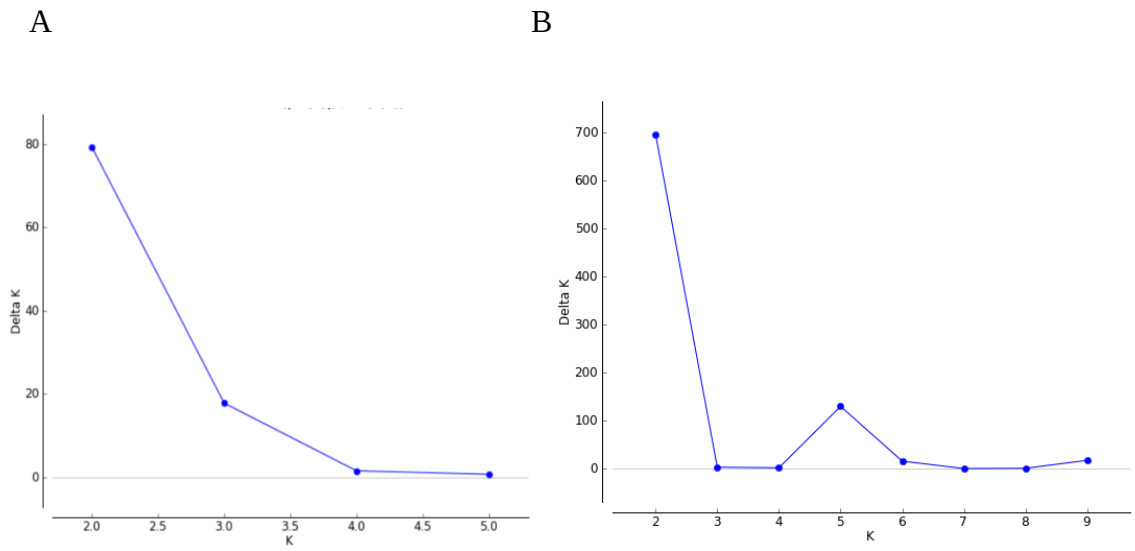


Figure 2 The rate of change in the STRUCTURE likelihood function (Delta or ΔK values) corrected for larger variance with increasing value of K as a function of the number of inferred clusters (K). The result suggests the BHW forms (a) two genetic population clusters (K=2) when the BHW sample was used alone, or (b) a single group (K=1) when included with samples of the RHW.

The STRUCTURE outputs and analysis is complex, and can seem a little daunting, so a brief explanation of interpreting the cluster analysis for the BHW is given in the Appendix (Appendix Figure 1).

The results suggest that the when considered together, the BHW and RHW form two (2) population clusters (Fig. 2), and correspond to a single population of the BHW and another with the RHW (Fig. 3). When only the BHW samples were included, they form two weak genetic population clusters (Fig. 3b). A further scrutiny of the cluster analysis by increasing the number of clusters reveals that with three inferred clusters, the two RHW groups are delineated (Fig. 3c). This pattern of a large difference between the RHW groups and the closer relationships between the BHW groups is also seen in Figs. 4-6.

An even larger inferred clustering (K=4; Fig 3d) shows that individuals from Dorre Island separate (shown in a red colour) from those on Bernier Island. At this resolution, the membership of individuals from the captive colonies become clearer, with those from Peron most likely to have been founded from individuals form Bernier Island. The RTD animals appear to have been founded from individuals sourced from Dorre Island.

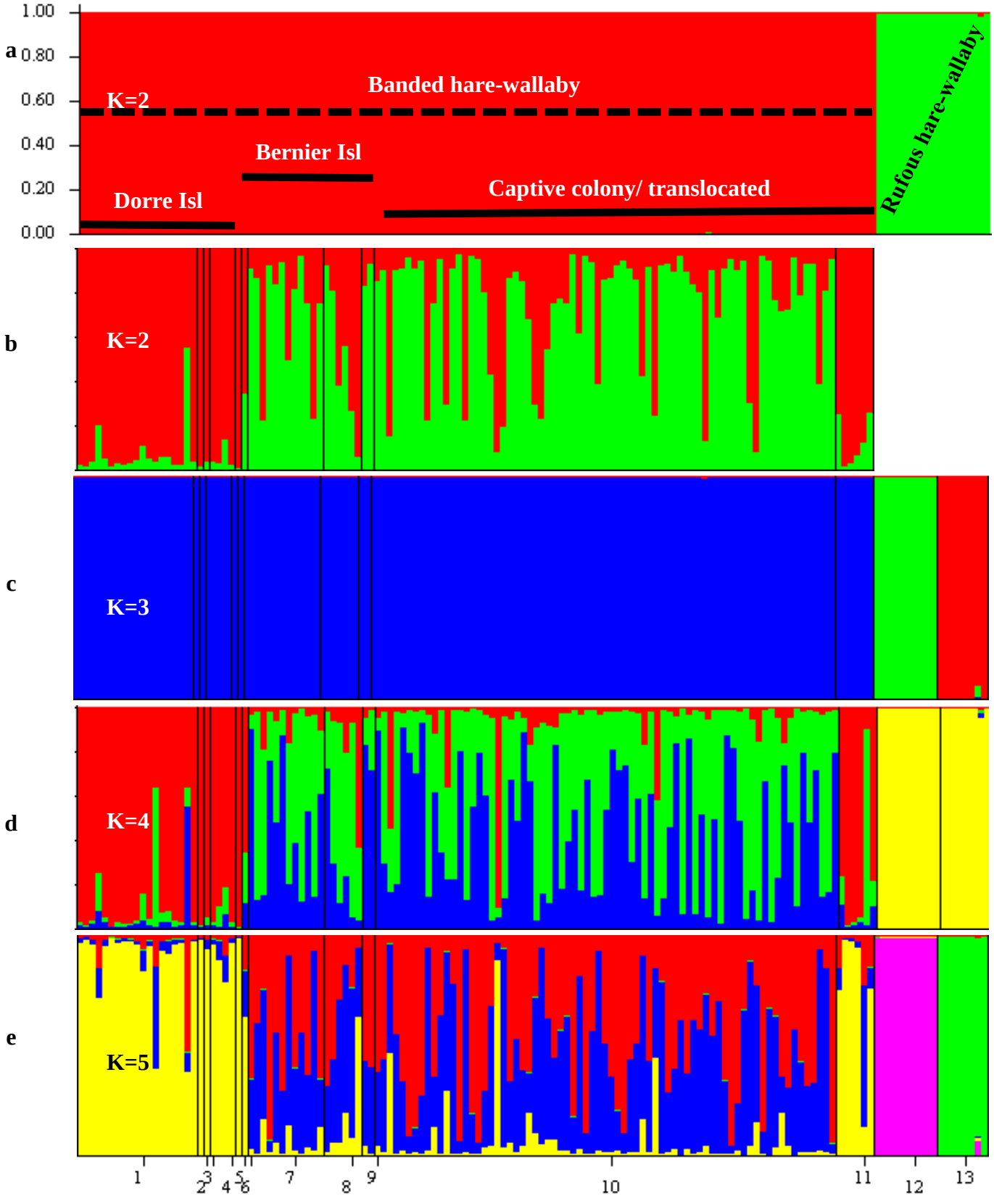


Figure 3. Bayesian population structure analysis. Bayesian assignment of the sampled populations, based on 10 nuclear microsatellite loci, assuming a population number of K = 2. Individuals are along the x-axis. The y-axis denotes the cumulative posterior probability of an individual's placement in particular population(s).

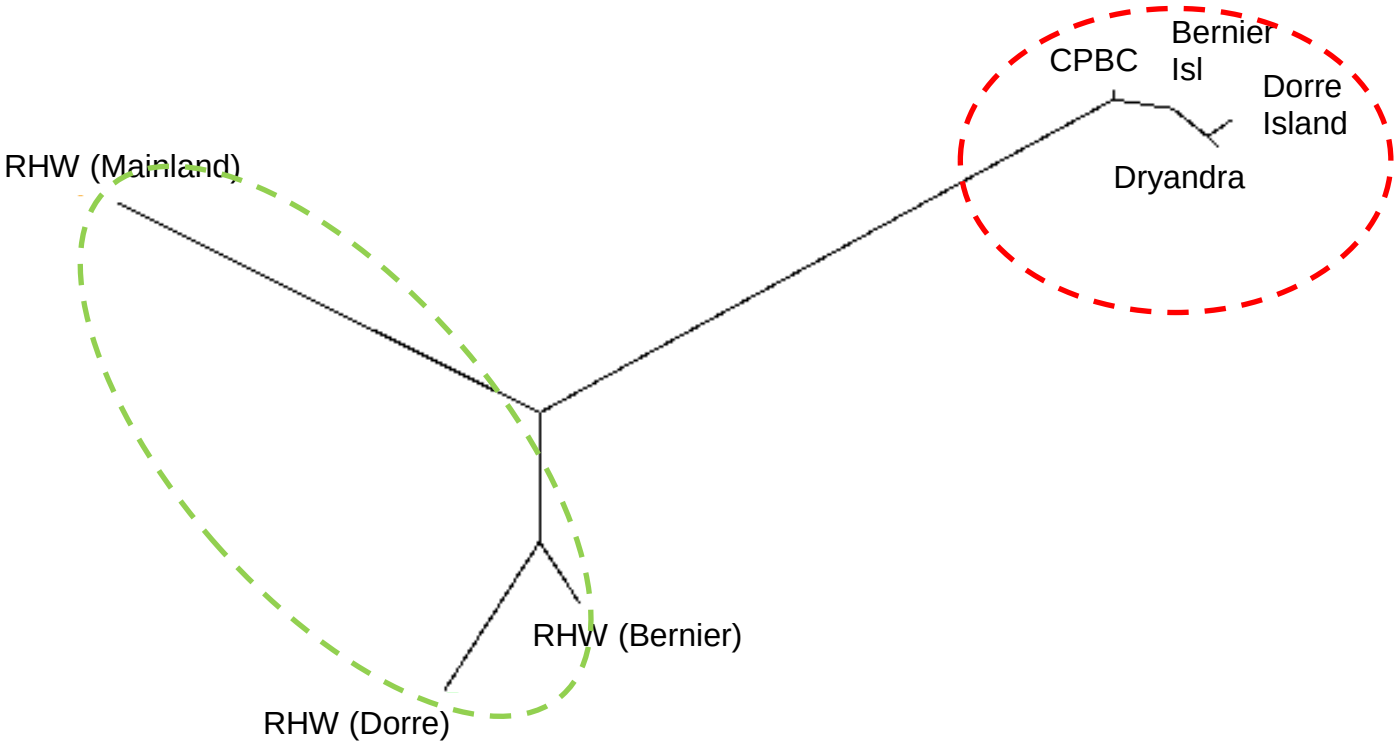


Figure 4. The tree show a representation of the genetic distances among the K=7 Structure clusters. The two main clusters are the BHW (circled in red), and the RHW (green).The trees are computed by applying the neighbour-joining algorithm to the matrix of allele-frequency divergence among clusters (net nucleotide distance). The tree was estimated using the program NEIGHBOR by Mary Kuhner and John Yamato, implementing Saitou and Nei's "Neighbor Joining Method"(Saitou and Nei 1987). The plot was produced using DRAWTREE (Felsenstein 2005)

The clustering analysis shows the best model is of two clusters. One cluster is of the banded and the other the rufous hare-wallabies. Not surprisingly, clusters show that the RHW were closely related (clustered together; Fig 3b-c).

Table 3. Percentage inferred ancestry (Q) of the averaged proportion of membership of each pre-defined population in each of the 2 clusters, when considering the BHW alone (shaded section), or 5 clusters when comparing genetic contribution from both the BHW and RHW. Values < 10% are not listed, and given as -

	BHW only		Inferred population cluster				
Given population	1	2	1	2	3	4	5
Bernier Is	92.3	-	44.7	46.2	-	-	-
Dorre Is	32.5	67.5	-	-	89.4	-	-
Captive PCBC	26.3	73.6	48.3	45.3	-	-	-
RTD	87.5	12.5	-	14.7	75.6	-	-
RHW Bernier Is	n/a	n/a	-	-	-	-	99.3
RHW Dorre Is	n/a	n/a	-	-	-	-	99.3
RHW Mainland	n/a	n/a	-	-	-	98.1	-

How unique are the hare-wallaby population? Differentiation among and within populations?

The BHW appears to form two weak genetic clusters (Fig. 2). The sample shows clear inter-relatedness between the sampled populations of the BHW, with no distinct clustering of discrete sampling points. This contrasts with the inclusion of samples from the RHW, which shows three clear clusters (Fig. 4), corresponding to a (i) single BHW cluster, (ii) mainland RHW and (iii) Bernier Island cluster of the RHW (Fig. 5).

The lack of a clear indication in the clustering pattern of the Dorre and Bernier Island samples (Fig. 2; Fig 3d,e; Table 4) is further illustrated by an intermixed pattern using a principle components analysis of the genetic distance between individuals (Fig. 6; Tables 4) in which no clear distribution can be made between the islands. This contrasts the clear patterns and differences occurring between the BHW and RHW (Fig. 5)

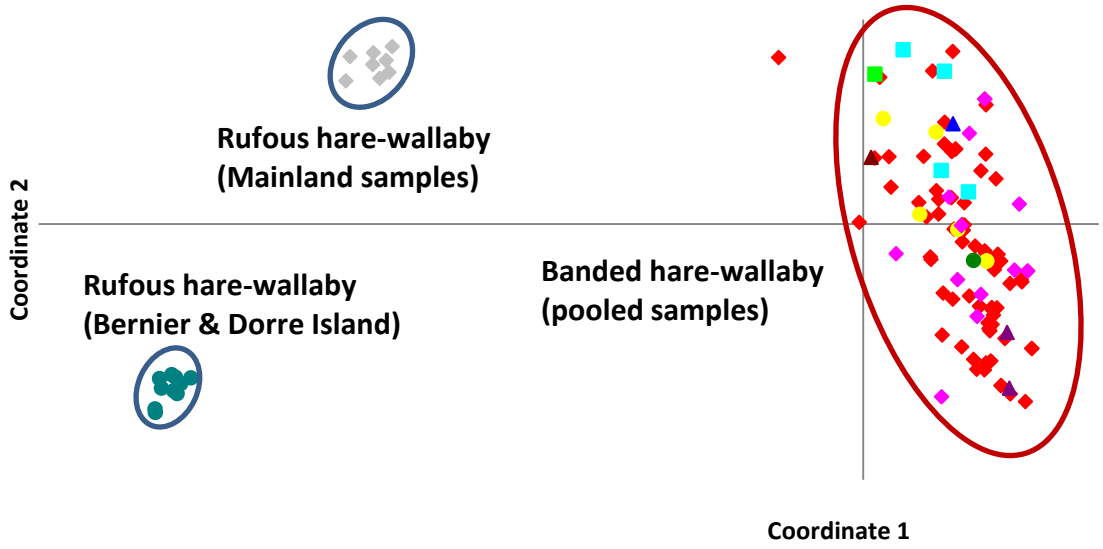


Figure 5. Principle components analysis based on a measure of the genetic distance (D_s) between sampling locations of hare-wallabies.

Table 4. The number of hare-wallabies that were assigned to their own population ("self-population") or clustered with another population ("Other pop").

Population	Self-Population	Other Pop	% assigned to another population
Banded hare-wallaby			
Bernier Is	6	15	71
Dorre Is	17	11	39
Captive bred			
Peron (PCBC)	32	41	57
Dryandra (RTD)	3	3	50
Rufous hare-wallaby			
Bernier Is	20	4 (DI)	None
Dorre Is	7	5 (BI)	40
Mainland	20	0	None
Total (Percentage, BHW only)	45 %	55%	

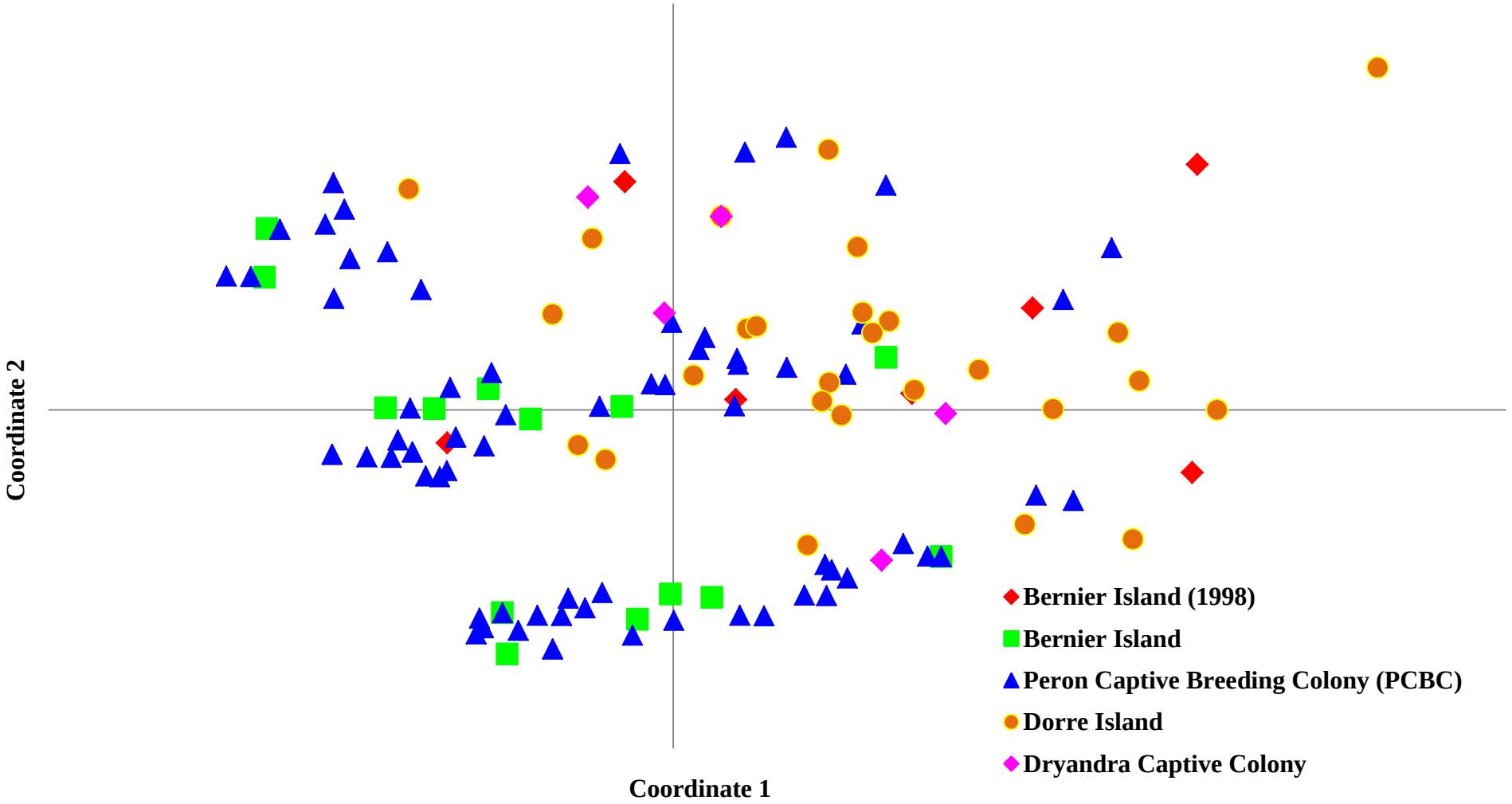


Figure 6. Principle components analysis of the genetic distance between sampling locations of the BHW.

The pooled estimates of F_{ST} (0.099 ± 0.021) between all the BHW were indicative of low levels of genetic differentiation amongst these populations (Table 5). Individual pair-wise F_{ST} values (Table 5) indicated moderate to low levels of differentiation between all pairs of populations (i.e. all values >0.1).

Estimates of genetic similarity (Table 6) identify that the Dorre Island population is identical to the RTD sample, and similarly, the Bernier Island population is identical to the PCBC.

Table 5. Pairwise F_{ST} estimates of population differentiation among BHW sampling sites, based upon the observed genotypes that were estimated from ten microsatellite loci. Values 0.05 – 0.15 indicate moderate genetic differentiation. Values less than 0.05 indicate very little genetic differentiation. All values were not significantly different from one another (P>0.05)

	Bernier (1998)	**Bernier Is**	**Captive bred (PCBC)**	**Dorre Is**	**Dryandra (RTD)**
Bernier (1998)	0.000				
Bernier Is	0.075	0.000			
PCBC	0.049	0.006	0.000		
Dorre Is	0.066	0.081	0.076	0.000	
RTD	0.059	0.065	0.062	0.019	0.000

Table 6. Pairwise estimates of Nei's (unbiased) genetic similarity among BHW sampling sites. All values were >95% similar to one another.

	Bernier (1998)	**Bernier Is**	**Captive bred (PCBC)**	**Dorre Is**	**Dryandra (RTD)**
Bernier (1998)	1				
Bernier Is	0.972	1			
PCBC	0.979	1.000	1		
Dorre Is	0.968	0.952	0.945	1	
RTD	0.983	0.973	0.965	1.000	1

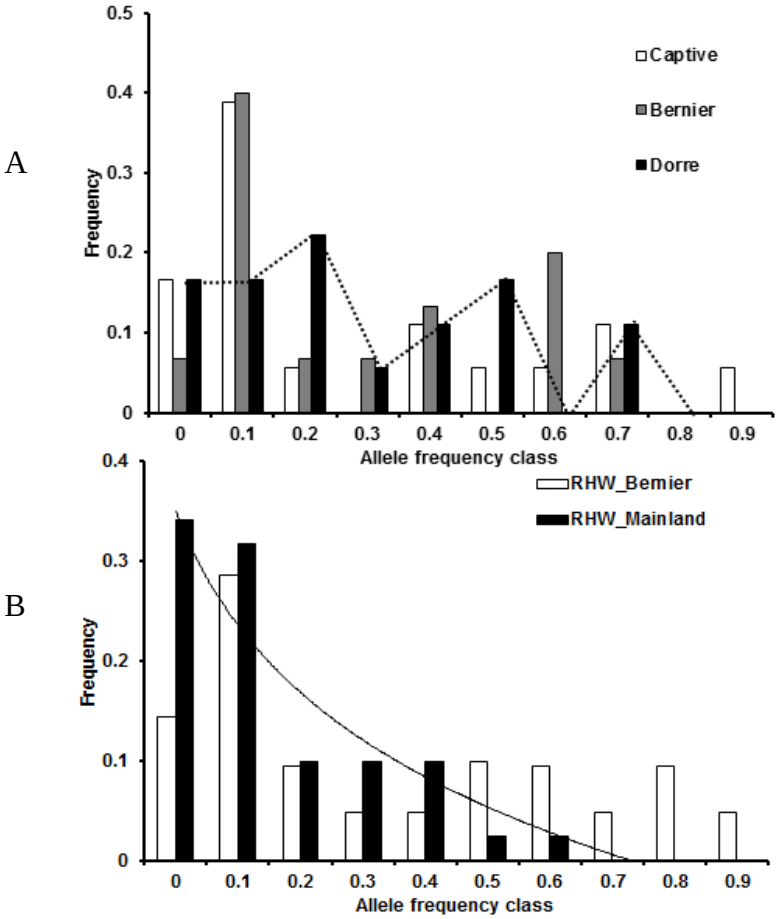


Figure 7. Distribution of allele frequency classes in the (a) BHW (b) and RHW from island (open bars) and mainland (filled bars) sites. The line in (b) represents the log best fit curve for the mainland RHW, and this distribution indicates no loss of rare alleles.

Under mutation-drift equilibrium, a non-bottlenecked population would show an L-shaped distribution, and as a bottleneck arises, the population tends to loose rarer alleles and the distribution becomes ‘distorted’. The distribution of allele classes in the BHW shows a distortion, and loss of rare alleles (Fig. 7). The Dorre Island population has experienced a recent genetic bottleneck, but no other BHW populations showed a genetic bottleneck, although all the island populations showed a shift in the allele frequency classes, suggesting a loss of rare alleles from the populations. The mainland population of the RHW showed allele class distribution that demonstrates a non-bottlenecked population (see the trend line in Fig. 7b). This relationship was not found in the other populations from the islands, for both BHWs and RHWs.

Individual heterozygosity. Individual heterozygosity was seen to be relatively low in all island populations, but increased on the mainland population of RHW (Fig. 8). This information identifies that the island populations general showed that less than 50% of individuals were heterozygous, suggesting that many individuals were genetically homozygous at these markers. This contrasts to the mainland RHW sample, where most individuals were heterozygous.

This data’s use may be most useful in prioritising which individuals to utilise in any further release programs. If the assumption that heterozygosity may reflect greater fitness in individuals, then individuals with more heterozygosity may allow more genetic ‘information’ (by way of carrying more than a single gene copy) to be captured in any subsequent translocation. It should be noted that individual heterozygosity reflects an individual’s bi-allelic combination and does not capture information on rare, or important genetic information contained in the population..

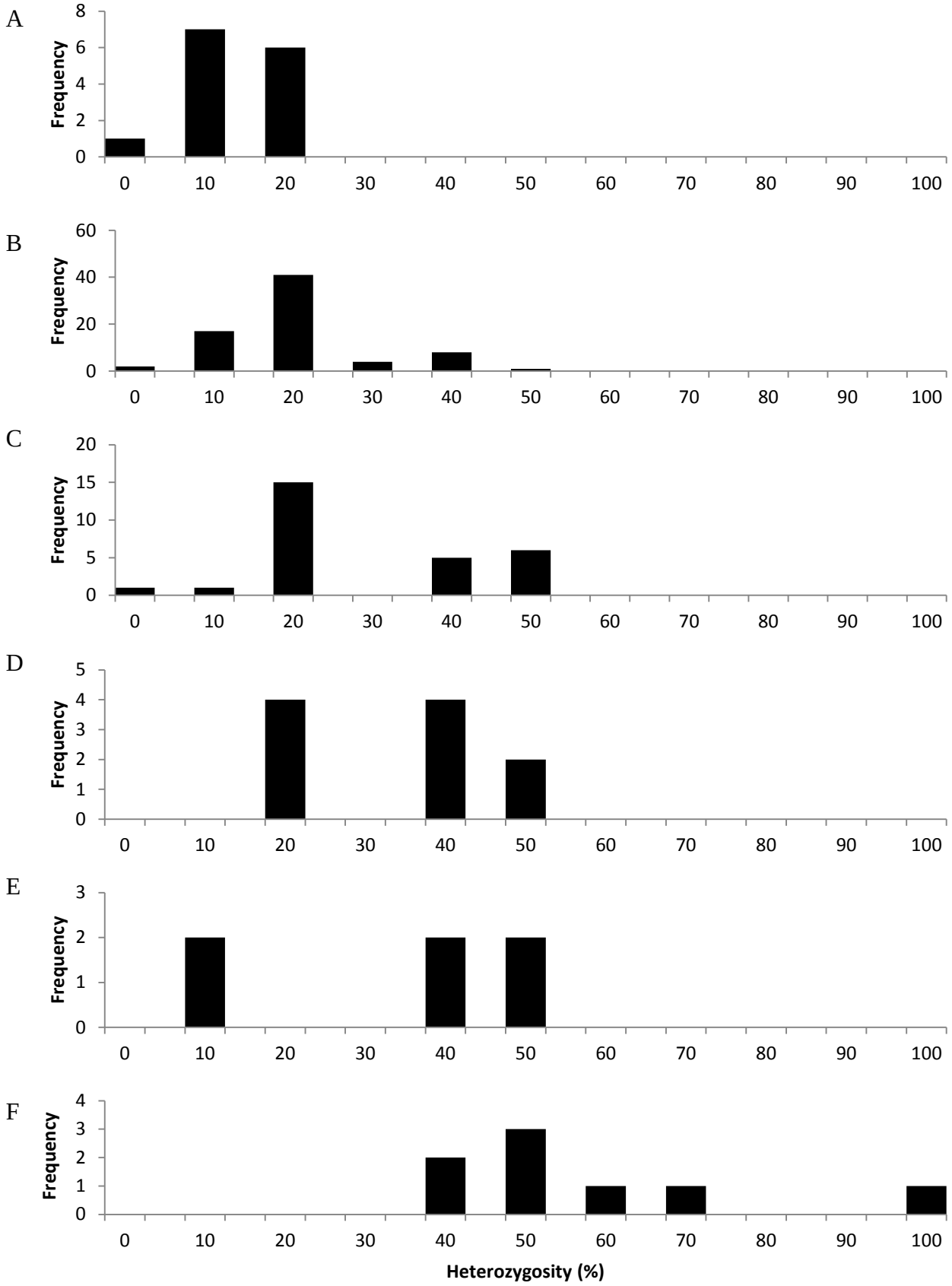


Figure 8. The frequency distribution of individual heterozygosity for each sampled population of BHW from (a) Dorre Isl, (b) Bernier Isl, (c) the Peron captive colony, (d) Dryandra colony and the (e) Bernier Island population of the RHW and (f) mainland RHW.

4. Discussion

Data from nuclear genetic markers show that there were poorly supported genetic differences between the sampled populations of the BHW. This finding is surprising, as it suggests that either both islands have followed very similar evolutionary trajectories (in terms of genetic drift, fitness, reproductive success etc.) or that there is some connectivity between Dorre and Bernier Islands, which are separated by 500m and open water.

The BHW results mirror the differences observed in island populations of the RHW and many other studies of marsupials marooned on islands. It is not really possible to draw absolute comparisons (because the BHW has no mainland counterparts), but it would again demonstrates the genetic importance of mainland stock.

The lack of any mainland source means that little can be done in the immediate future to increase the genetic diversity in prevailing translocated populations of the BHW. The most prudent outcome would be to continue to mix stock from both the islands and used this admixed stock as founders or additions to the existing captive breeding program.

Genetic distances/divergence (based on microsatellite data) amongst BHW populations are low. Overall, the results show that the individuals in the Peron captive breeding colony represent nearly all the genetic information available from the source population (Bernier Island).

Island and fragmented populations are known to be susceptible to inbreeding, which often results in the loss of genetic diversity and inbreeding depression (Frankham *et al.* 2009). The consequences of increased homozygosity for individual fitness has been shown repeatedly within captive populations, leading to increased neonatal and juvenile mortality (Ralls *et al.* 1979) as well as compromised reproduction (Fredrickson *et al.* 2007) and longevity. To provide some guidance to the decision process for which individuals would be best for introduction onto Dirk Hartog Island, we provide a ‘ranked’ list of individual heterozygosity. There are a number of different approaches to measuring heterozygosity (with pros and cons for each), so we provide a list in the supplementary section (Appendix Table 2) that ranks from most down to least-heterozygote individuals genotyped in this study. If diversity is perceived as an important attribute in choosing individuals for the translocation, it should be a

relatively straightforward step to choose which individuals, from any chosen source population as a founder for the new translocation program.

For the RHW, as expected each island population basically retains a small subset of alleles present in the mainland. They are not substantially different from each other or the mainland population. The remnant mainland RHW population (now in captivity) is an absolute treasure. It retains high levels of genetic variation and there is no genetic evidence that viability will be lost in the long-term. However to prevent the loss of this variation it is imperative that the current captive population be rapidly increased in size (preferable into the thousands) as the current population size is too small to preserve the genetic diversity this population currently contains. From a genetic viewpoint, the captive population should be used to source all reintroduction efforts. The island populations are inbred, have low variation and at this stage should just be left to "muddle on".

This work clearly demonstrates that population-level genetic research is highly beneficial in aiding management decisions for the recovery process. The outcomes are not only (relatively) inexpensive, but molecular techniques are highly productive tools for management of endangered species.

On a cautionary final note, the historical relationships of the managed populations should be considered because conservation programs may mix populations with no historical connection, and thereby homogenise prior subpopulation differentiation. This may ultimately swamp local adaptation and/or homogenise fixed genetic differences. It may also lead to continued introgression, preventing future local adaptation. Finally, our inability to predict future environmental change presents a problem with enhancing gene flow. Continued introduction of populations that are poorly adapted may prevent future local adaptation.

Translocation proposal to Dirk Hartog Island – Recommendations

There appear to be three options in relation to the proposed translocation to Dirk Hartog Island.

1. **Utilise animals from the Peron Captive Breeding Colony**. One option would be to keep the status quo and simply use the existing captive colony as founders for the translocation operation. This group of animals encapsulates the genetic composition of its founding population (from Bernier Island) and would in essence, replicate the diversity and genetics of that island.

This is not an option, as the PCBC was closed and all BHW were relocated to Faure Is or Wadderin (in the wheatbelt; -31.9954 lat.; 118.4457 long.)

2. **Utilise animals from the Dorre Island**. At present there are no surviving individuals of 'Dorre Island BHW' anywhere except on Dorre Island. A second option would be to utilise and form an insurance population of these animals. From a genetic perspective this would not achieve any greater consequence for the species, as individuals are less diverse than individuals from Bernier Island, or its duplicated colony on the mainland. The Dorre Island group of animals would fail to capture all the available genetic information in the species as a result of its lower genetic diversity indices.

3. **Use a combination of animals from Dorre and Bernier Island.** The final option would be to combine individuals from both the islands as founders for the translocation operation. This group of animals would ensure persistence of the Dorre Island animals and encapsulates the entire available genetic configuration of its founding population (from Bernier Island) and would in essence, replicate the diversity and genetics of both the remaining island.

5. References

Abbott I. (2000). Improving the conservation of threatened and rare mammal species through translocation to islands: case study Western Australia. Biological Conservation, 93: 195-201

Abbott I., Burbidge, A.A. (1995) The occurrence of mammal species on the islands of Australia: a summary of existing knowledge. CALMScience,1: 259–324

Amos W., Worthington Wilmer J., Fullard K., et al. (2001). The influence of parental relatedness on reproductive success. Proceedings of the Royal Society B: Biological Sciences. 268: 2021–2027.

Aparicio J.M., Ortego J., Cordero P.J. (2006). What should we weigh to estimate heterozygosity, alleles or loci? Molecular Ecology 15: 4659–4665.

Atkinson I.A.E. (2001). Introduced mammals and models for restoration. Biological Conservation 99, 81–96.

Avise J. (1994). Molecular markers, natural history and evolution. Chapman and Hall, New York.

Burbidge A.A. (1999). Conservation values and management of Australian islands for non-volant mammal conservation. Australian Mammalogy, 21: 67–74.

Burbidge A.A, Manly B.J.F (2002). Mammal extinctions on Australian islands: causes and conservation implications. Journal of Biogeography 29:465–473.

Burbidge A.A., N.L. McKenzie (1989). Patterns in the modern decline of Western Australia's vertebrate fauna: causes and conservation implications. Biological Conservation, 50: 143–198

Burbidge A.A., Williams M.R., Abbott I. (1997). Mammals of Australian islands: Factors influencing species richness. Journal of Biogeography 24: 703–715.

Cockerham C.C, Weir, B.S. (1993). Estimation of gene flow from F-statistics. Evolution, 47: 855-863.

Coltman D.W., Pilkington J.G., Smith J.A., Pemberton J.M. (1999). Parasite-mediated selection against inbred Soay sheep in a free-living, island population.Evolution 53, 1259–1267.

Eldridge M.D.B., King J.M., Loupis A.K., Spencer P.B.S., Taylor A.C., Pope L.C., Hall G.P. (1999). Unprecedented low levels of genetic variation and inbreeding depression in an island population of the black-footed rock-wallaby. Conservation Biology, 13: 531–541.

Evanno G,. Regnaut, S. Goudet, J. (2005). Detecting the number of clusters of individuals using the software structure: a simulation study. Molecular Ecology 14, 2611–2620.

Felsenstein J. (2005). PHYLIP (Phylogeny Inference Package) version 3.6. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle.

Frankham, J. D. Ballou, D. A. Briscoe. (2009). Introduction to conservation genetics, 2nd edition, Cambridge University Press, Cambridge.

Fredrickson, R.J., Siminski, P., Woolf, M. et al. (2007). Genetic rescue and inbreeding depression in Mexican wolves. Proceedings Of The Royal Society B-Biological Sciences, 274: 2365-2371

Friend, T., B. Beecham (2004). Return to Dryandra: Western Shield. Conservation Science Western Australia, 5: 174-193.

Goudet, J. (1995). FSTAT: a computer program to calculate F-statistics. Journal of Heredity 86:485-486.

Gower, J.C. (1966). Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika, 53: 325-338.

Ihaka R., R. Gentleman (1996). R: a language for data analysis and graphics. Journal of Computing Graph. Statistics, 5: 299–314

Kalinowski, S.T. (2006). HW-QuickCheck: an easy-to-use computer program for checking genotypes for agreement with Hardy–Weinberg expectations. Molecular Ecology Notes, 6: 974 -979.

Luikart, G., Cornuet, J-M. (1998) Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. Conservation Biology 12: 228-237.

Menkhorst, P. (2001). A Filed Guide to the Mammals of Australia. South Melbourne, Australia: Oxford University Press.

Mills H. R., Moro D., Spencer P.B.S. (2004). Conservation significance of island versus mainland populations: a case study of dibblers (*Parantechinus apicalis*) in Western Australia. Animal Conservation 7: 387-395.

Moro D. (2003). Translocation of captive-bred dibblers *Parantechinus apicalis* (Marsupialia: Dasyuridae) to Escape Island, Western Australia. Biological Conservation 111: 305-315.

Richards, J., Morris, K., Burbidge, A., Friend, T. (2008). *Lagostrophus fasciatus*. In: IUCN 2008. IUCN Red List of Threatened Species. Retrieved 28 December 2008.

Richards, J., J. Short, R. Prince, J. Friend, J. Courtenay. (2001). The biology of banded (*Lagostrophus fasciatus*) and rufous (*Lagorchestes hirsutus*) hare-wallabies (Diprotodontia: Macropodidae) on Dorre and Bernier Islands, Western Australia. Wildlife Research, 28: 311-322.

Peakall, R., Smouse, P.E. (2006). GenAlEx 6: genetic analysis in Excel. genetics software for teaching & research. Molecular Ecology Notes, 6: 288-295.

Piry S., Luikart G., Cornuet J.M. (1999). BOTTLENECK: A computer program for detecting recent reductions in the effective population size using allele frequency data. Journal of Heredity 90: 502-503.

Pope L, Sharp A & Moritz C (1996). Population structure of the yellow-footed rock-wallaby *Petrogale xanthopus* (Gray, 1854) inferred from mtDNA sequences and microsatellites. Molecular Ecology, 5: 629–640.

Prideaux, G. J., Warburton, N. M. (2010). An osteology-based appraisal of the phylogeny and evolution of kangaroos and wallabies (Macropodidae: Marsupialia), Zoological Journal of the Linnean Society, 159: 954–87.

Pritchard, J.K., Stephens, M., Donnelly, O. (2000). Inference of population structure using multilocus genotype data. Genetics 155: 945-959.

Ralls K., K. Brugger, J. Ballou (1979). Inbreeding and juvenile mortality in small populations of ungulates. Science, 206:1101-1103.

Raymond, M., Rousset, F. (1995). GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity 86: 248-249.

Ricciardi A., D. Simberloff (2009). Assisted colonization is not a viable conservation strategy. Trends in Ecology & Evolution 24, 248–253

Richards, J., Morris, K., Burbidge, A. & Friend, T. (2008). *Lagostrophus fasciatus*. In: IUCN 2008. IUCN Red List of Threatened Species.

Saitou N, Nei M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4:406-25.

Serena, M., (ed.) 1995. Reintroduction biology of Australian and New Zealand fauna. Surrey Beatty, Sydney.

Short, J., B. Turner. (1992). The distribution and abundance of the banded and rufous hare-wallabies *Lagostrophus fasciatus* and *Lagorchestes hirstus*. Biological Conservation, 60: 157-166.

Slatkin M. (1987). Gene flow and the geographical structure of natural populations. Science 236: 787-792.

Taylor AC, Cooper DW (1998). A set of tammar wallaby (*Macropus eugenii*) microsatellites tested for genetic linkage. Molecular Ecology 7: 925-926.

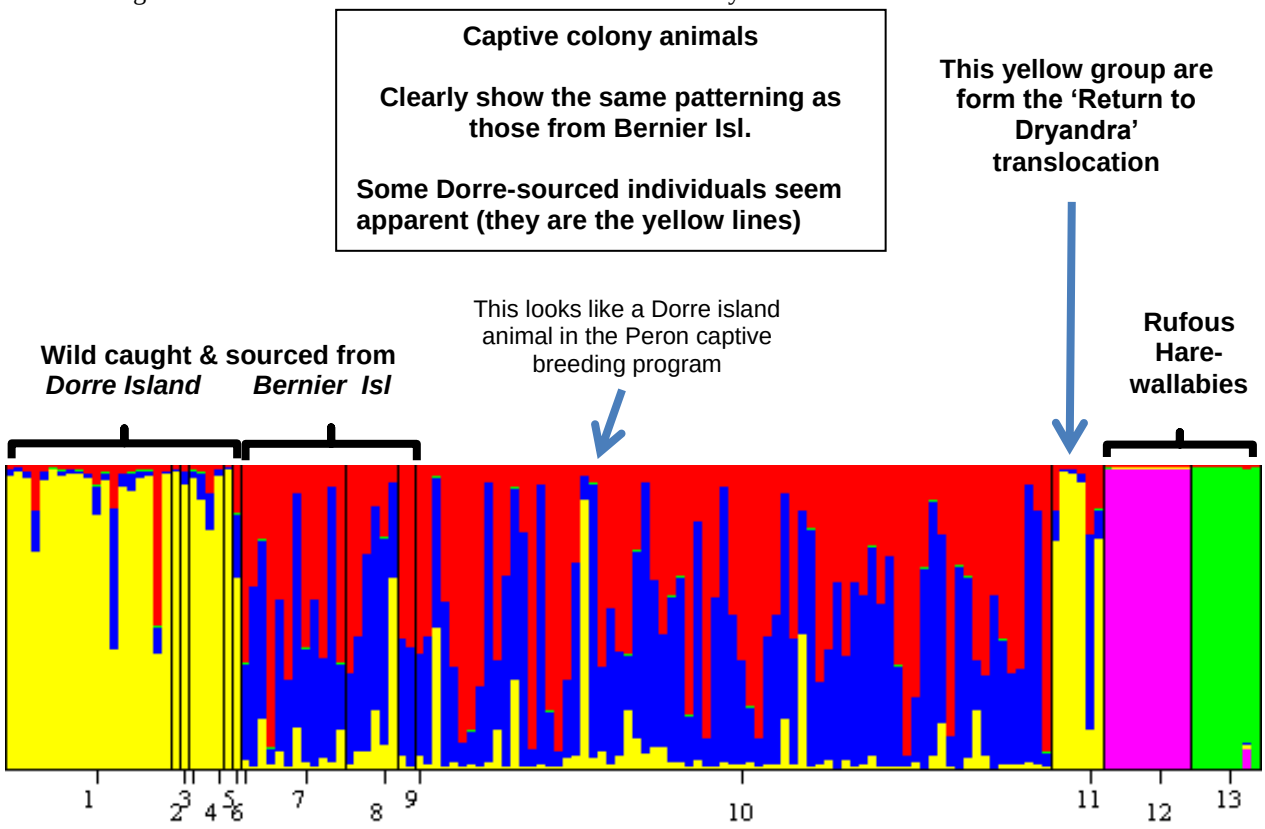
Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M., Shipley, P. (2004). MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4: 535–538.

Waples, R.S., Do, S. (2008). LDNE: a program for estimating effective population size from data on linkage disequilibrium. Molecular Ecology Notes 8: 753–756.

Weir, B. S., Cockerham, C. C. (1984) Estimating F-statistics for the analysis of population structure. Evolution 38: 1358 - 1370.

Williams G.R. (1977). Marooning — a technique for saving threatened species from extinction. International Zoo Yearbook, 17: 102–106.

Wright, S. (1931) Evolution of Mendelian populations. Genetics 16: 97 - 159.



Population (sampled)	Colour on figure	Number of figure	Number of individuals
Dorre Island			
White Beach	yellow	1	20
Castle	yellow	2	1
Disaster Cove	yellow	3	1
Pinnacle	yellow	4	4
Quion Bluff	yellow	5	1
South	red/blue	6	1
Bernier Island			
Hospital Bay	red/blue	7	12
1998 samples	red/blue	8	7
unknown*	red/blue	9	2
Captive breeding stock			
Peron (Captive breeding colony)	red/blue	10	73
Dryandra captive colony	yellow	11	6
Rufous hare wallaby			
Bernier	pink	12	10
Mainland	green	13	8

Appendix Figure 1 A brief explanation of the STRUCTURE figures. This figure is generated by a program called STRUCTURE. It groups genetically similar individuals together. In the example given, there is 5 different 'populations' (denoted by the different colours). The populations, in this case, correspond closely to the sampling locations (in the table). Each individual hare-wallaby in the analysis is shown as a vertical bar. The Y-axis is best described as its 'genetic uniqueness', where by if the bar is just one colour (see the pink RHW samples, for instance, then it suggests that they are 100% RHW, with no other genetic 'pollution' from another population. The more interesting samples are those that 'share' colours. By way of example, the last individual from the Dryandra sample is probably ~70% Dorre Is, but has some blue/red (characteristic of an individual from Bernier Is). If it was 50:50 (Dorre: Bernier Isl), it would suggest that the individual had one parent from each island (i.e. admixed). In general though, the Dryandra animals are yellow (suggesting a Dorre Is source), and the Peron animals appear to be from Bernier Is.

Appendix Table. 1 Observed allele frequencies, at 10 microsatellite loci, in sampled banded hare-wallabies and rufous hare-wallaby populations.

	Banded hare-wallaby											Rufous hare-wallaby		
Locus	**Dorre Island**						**Bernier Island**			**Captive colonies**				
Pa593	**White Beach**	**Castle**	**Disaster Cove**	**Pinnacle**	**Quoin Bluff**	**South**	**HospitalBay**	**Unknown**	**Bernier**	**PCBC**	**Dryandra**	**Dorre**	**Bernier**	**Captive**
93	3.3										8.3			
95	53.3	100.0		62.5	41.7	50.0	29.2		16.7	13.0	58.3			
97							16.7		16.7	11.1				
99							12.5	25.0		9.3				
101	10.0						8.3		16.7	17.3	8.3			
103	20.0		50.0	25.0	41.7	50.0	33.3	75.0	50.0	49.4	16.7			
105	13.3		50.0	12.5	16.7						8.3			
107														8.3
111														44.4
115														8.3
117													87.5	
119													12.5	5.6
127												25.0		
129												75.0		
131														33.3
Me14														
155	25.0	100.0		25.0	33.3		12.5	25.0	25.0	17.3	25.0			
157	75.0		100.0	75.0	66.7	100.0	79.2	75.0	75.0	66.0	75.0			
159							8.3			16.7				
167													12.5	
177													8.3	
179												100.0	72.9	
183													4.2	

185													2.1	
195														20.0
197														45.0
199														15.0
201														10.0
203														5.0
205														2.5
207														2.5
y105														
219	14.3				33.3						25.0			
223	46.4	100.0	50.0	50.0	41.7		70.8	25.0	50.0	56.1	25.0			
231	35.7		50.0	50.0	16.7	100.0	29.2	75.0	50.0	43.9	50.0			
235	3.6				8.3									
243												33.3		
249												66.7	100.0	
253														21.4
265														32.1
267														46.4
Pa385														
155	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Pa297														
107														100.0
115	42.3	50.0		12.5	16.7					0.6	8.3			
117	53.8		100.0	75.0	41.7	100.0	100.0	100.0	100.0	99.4	83.3			
119	3.8	50.0		12.5	41.7						8.3			
129												66.7		
133												33.3	95.7	
135													4.3	

y175														
264													58.3	7.5
268	53.8	50.0		12.5	50.0	50.0	79.2	100.0	35.7	79.5	50.0			7.5
274	46.2	50.0	100.0	87.5	50.0	50.0	20.8		64.3	20.5	50.0			
276														12.5
280														17.5
282												75.0	41.7	20.0
284												16.7		10.0
286														17.5
288														7.5
304												8.3		
y151														
142	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0			
158														13.9
160														41.7
162														16.7
164														8.3
172													6.3	
174												91.7	64.6	
176													29.2	
178												8.3		
180														8.3
182														11.1
Me17														
101	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0			
133													8.3	
135													52.1	15.0
137													14.6	
145												91.7		25.0
147												8.3	25.0	

149														12.5
151														25.0
159														5.0
161														12.5
163														5.0
y148														
154	60.7	100.0	50.0	75.0	91.7	50.0	72.7	100.0	37.5	70.1	75.0			
156										0.6				
158	39.3		50.0	25.0	8.3	50.0	13.6		37.5	16.2	25.0			
160							13.6		25.0	13.0				
168												100.0		
170														32.5
178														15.0
180													10.4	17.5
182													89.6	
186														30.0
188														5.0
Pa55														
145	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0			
163													70.8	
167												100.0	4.2	13.9
169														22.2
171													25.0	63.9

Appendix Table 2. A complete list of each genotyped individual Banded hare-wallaby ranked from the most heterozygous to least heterozygous individuals sampled.

PHt proportion of heterozygous loci () in an individual: PHt = number of heterozygous loci / number of genotyped loci

Hs_exp standardized heterozygosity based on the mean expected heterozygosity (, Coltman 1999): Hs_exp = PHt / mean expected heterozygosity of typed loci.

IR internal relatedness () (Amos 2001): IR = (2H – Σ fi) / (2N – Σ fi), where H is the number of loci that are homozygous, N is the number of loci and fi is the frequency of the i^{th} allele contained in the genotype. The maximum value (+1) is obtained when all loci are homozygous, regardless of allelic frequencies; whereas the minimum value (-1.0) can be obtained only when all loci present only two alleles and the individual is heterozygous for all of them.

HL homozygosity by locus () (Aparicio 2006): HL = Σ Eh / (Σ Eh + Σ Ej), where Eh and Ej are the expected heterozygosities of the loci that an individual bears in homozygosis (h) and in heterozygosis (j), respectively. The larger the number, the MORE homozygous the genotype is. Therefore, the smaller the number, the more heterozygous the individual.

Sample	ID No. or microchip	Heterozygosity measures				Location	Date	Sex
		PHt	Hs_exp	IR	HL*			
13-145	Dryandra2	0.600	2.130	-0.627	0.000	Return to Dryandra - South Enclosure	24/08/2000	M
13-190	Blossom	0.500	1.840	-0.491	0.000	captive bred PCBC	13/12/2008	F
13-262	0006E22B8E	0.600	2.130	-0.718	0.000	Dorre Is. White Beach	14/08/1996	M
13-226	BH241	0.444	1.684	-0.462	0.081	captive bred PCBC		M
13-146	Dryandra3	0.500	1.775	-0.515	0.160	Return to Dryandra - South Enclosure	23/08/2000	M
13-232		0.500	1.775	-0.539	0.160	Dorre Island X Kuhn	8/06/2013	?
13-236		0.500	1.775	-0.630	0.160	Dorre Island Quoin	8/06/2013	?
13-263	0	0.500	1.775	-0.515	0.160	Dorre Is. White Beach	15/08/1996	M
13-271		0.500	1.775	-0.539	0.160	Dorre Is. White Beach	6/09/1999	M
13-217	BH257	0.400	1.420	-0.261	0.225	captive bred PCBC		F
13-147	Dryandra4	0.400	1.420	-0.383	0.231	Return to Dryandra - South Enclosure	17/12/1999	F

13-260		0.400	1.420	-0.024	0.231	Dorre Is. White Beach	13/08/1995	F
13-148	Dryandra5	0.400	1.420	-0.297	0.260	Return to Dryandra - South Enclosure	6/04/2002	M
13-203		0.400	1.420	-0.351	0.260	captive bred PCBC	27/06/2006	F
13-229	0006E231F3	0.400	1.420	-0.374	0.260	captive bred PCBC		M
13-207	BH119	0.400	1.420	-0.345	0.260	captive bred PCBC	19/10/2006	M
13-209	BH116	0.400	1.420	-0.362	0.260	captive bred PCBC	26/07/2006	F
13-336	BH261	0.400	1.420	-0.374	0.260	captive bred PCBC	26/08/2013	M
13-261	0006E21FE0	0.500	1.775	-0.478	0.261	Dorre Is. White Beach	14/08/1996	F
13-228	0006E240ED	0.333	1.263	-0.242	0.271	captive bred PCBC		M
13-332	BH157	0.333	1.263	-0.351	0.271	captive bred PCBC		M
13-212	ear tag 787	0.333	1.263	-0.343	0.274	captive bred PCBC	18/09/2007	M
13-205		0.333	1.268	-0.340	0.276	captive bred PCBC	30/11/2006	M
13-195		0.444	1.691	-0.493	0.310	captive bred PCBC	11/09/2006	M
13-234		0.400	1.420	-0.262	0.317	Dorre Island Quoin	8/06/2013	?
13-274		0.400	1.420	-0.204	0.320	Dorre Is. White Beach	20/09/2000	F
13-266	7	0.400	1.420	-0.204	0.323	Dorre Is. White Beach	19/09/1996	F
13-180	0006E23B13	0.300	1.065	-0.152	0.385	captive bred PCBC	15/11/2011	M
13-202	Jasmin	0.300	1.065	-0.216	0.385	captive bred PCBC	16/11/2006	F
13-215	BH248	0.300	1.065	-0.176	0.385	captive bred PCBC		F
13-223	BH245	0.300	1.065	-0.084	0.385	captive bred PCBC		M
13-331	BH175	0.300	1.065	-0.137	0.385	Bernier Hospital Bay		F
13-045		0.333	1.065	-0.175	0.388		30/10/1998	F
13-052		0.300	1.065	0.190	0.388		8/10/1998	M
13-044	BH23	0.300	1.065	-0.044	0.388		9/10/1998	M
13-185	0006E4CC31	0.300	1.065	-0.148	0.388	captive bred PCBC		f
13-188	Babel	0.300	1.065	-0.114	0.388	captive bred PCBC	16/10/2007	m
13-198		0.300	1.065	-0.171	0.388	captive bred PCBC	7/06/2006	M
13-227	0006??3804	0.300	1.065	-0.158	0.388	captive bred PCBC		M
13-270		0.300	1.065	0.000	0.388	Dorre Is. White Beach	6/09/1999	F
13-275		0.300	1.065	-0.027	0.388	Dorre Is. Disaster Cove	10/03/2003	F
13-192	BH152	0.300	1.065	-0.185	0.388	captive bred PCBC	12/12/2008	F

13-221	BH258	0.300	1.065	-0.158	0.388	captive bred PCBC		F
13-334	BH260	0.300	1.065	-0.175	0.388	captive bred PCBC		F
13-339	BH259	0.300	1.065	-0.114	0.388	captive bred PCBC	26/08/2013	F
13-168	0006E4D924	0.300	1.065	-0.047	0.416	Bernier Hospital Bay	11/08/2010	M
13-230	0006E231A3	0.300	1.065	-0.098	0.416	captive bred PCBC		F
13-164	BH182	0.300	1.065	-0.042	0.416	captive bred PCBC	27/07/2011	
13-218	BH196	0.300	1.065	-0.022	0.416	captive bred PCBC		F
13-269		0.400	1.420	-0.151	0.421	Dorre Is. White Beach	5/09/1999	M
13-047		0.333	1.065	-0.030	0.423		6/11/1998	F
13-214	0006E246F1	0.300	1.065	-0.130	0.423	captive bred PCBC		F
13-231		0.300	1.065	-0.107	0.423	Dorre Island South	7/06/2013	?
13-175	BH195	0.300	1.065	-0.166	0.423	captive bred PCBC	15/01/2012	F
13-244		0.222	0.842	-0.014	0.464	captive bred PCBC	13/07/2013	M
13-206	BH121	0.222	0.842	-0.010	0.464	captive bred PCBC	16/11/2006	F
13-225	BH255	0.222	0.842	0.015	0.464	captive bred PCBC		M
13-183	0006E24161	0.300	1.065	0.151	0.486	Bernier Hospital Bay	1/07/2011	M
13-233		0.300	1.065	0.254	0.486	Dorre Island Quoin	8/06/2013	?
13-240		0.300	1.065	-0.104	0.489	Dorre Island Pinnacle	9/06/2013	?
13-049	BH17	0.222	0.845	0.183	0.496			F
13-189	Petal	0.222	0.842	0.118	0.498	captive bred PCBC	13/12/2008	F
13-211	ear tag 786	0.222	0.842	0.063	0.501	captive bred PCBC	18/09/2007	M
13-267		0.300	1.065	0.123	0.511	Dorre Is. White Beach	2/09/1999	M
13-268		0.300	1.065	-0.097	0.514	Dorre Is. White Beach	3/09/1999	F
13-197	charlotte	0.143	0.853	-0.042	0.541	captive bred PCBC	18/01/2007	F
13-169	0006E4C7F7	0.200	0.710	0.266	0.548	Bernier Hospital Bay	11/08/2010	F
13-159	0006E	0.200	0.710	0.235	0.576	captive bred PCBC		
13-186	Alfouse	0.200	0.710	0.362	0.576	captive bred PCBC	15/11/2007	M
13-160	BH166	0.200	0.710	0.230	0.576	captive bred PCBC	25/01/2011	F
13-201	BH111	0.200	0.710	0.472	0.576	captive bred PCBC	24/05/2006	F
13-224	BH250	0.200	0.710	0.211	0.576	captive bred PCBC		M
13-196		0.222	0.845	0.270	0.578	captive bred PCBC	11/09/2006	M

13-157	0006E23508	0.200	0.710	0.188	0.579	captive bred PCBC	23/08/2010	F
13-176	0006E24DE6	0.200	0.710	0.187	0.579	captive bred PCBC	18/01/2012	M
13-187	Beazley	0.200	0.710	0.207	0.579	captive bred PCBC	15/11/2007	M
13-161	BH181	0.200	0.710	0.238	0.579	captive bred PCBC	27/07/2011	M
13-163	BH169	0.200	0.710	0.188	0.579		11/08/2010	F
13-174	BH194	0.200	0.710	0.240	0.579	captive bred PCBC	18/01/2012	F
13-216	BH253	0.200	0.710	0.276	0.579	captive bred PCBC		F
13-222	BH242	0.200	0.710	0.382	0.579	captive bred PCBC		M
13-272		0.200	0.710	0.483	0.583	Dorre Is. Quoin Bluff	9/09/1999	F
13-265	0006E236B2	0.286	0.916	0.128	0.583	Dorre Is. White Beach	19/08/1996	F
13-002	BBH1 (OR BBH11)	0.222	0.845	0.009	0.586			
13-172	0006E241EF	0.200	0.710	0.263	0.646	Bernier Hospital Bay	25/01/2011	F
13-239		0.200	0.710	0.410	0.646	Dorre Island Pinnacle	9/06/2013	?
13-242		0.200	0.710	0.162	0.646		29/07/2009	F
13-167	0006E	0.200	0.710	0.184	0.649	captive bred PCBC	12/05/2010	M
13-191	BH154	0.200	0.710	0.332	0.649	captive bred PCBC	12/12/2008	M
13-182	0006E24D2D	0.200	0.710	0.287	0.652	captive bred PCBC	15/11/2011	M
13-235		0.200	0.710	0.190	0.652	Dorre Island Quoin	8/06/2013	?
13-238		0.200	0.710	0.343	0.671	Dorre Island Pinnacle	9/06/2013	?
13-241		0.200	0.710	0.340	0.671	Dorre Island Pinnacle	9/06/2013	?
13-337	BH168	0.200	0.710	0.304	0.677	captive bred PCBC	26/08/2013	M
13-165	BH183	0.200	0.710	0.198	0.683	captive bred PCBC	27/07/2011	M
13-181	0006E241EE	0.111	0.423	0.507	0.690	Bernier Hospital Bay	1/07/2011	F
13-177	0006E23334	0.100	0.355	0.578	0.739	captive bred PCBC	15/11/2011	M
13-178	0006E22B84	0.100	0.355	0.613	0.739		1/07/2011	F
13-179	0006E23DB2	0.100	0.355	0.535	0.739		1/07/2011	F
13-243		0.100	0.355	0.580	0.739		29/07/2009	M
13-208	BH124	0.100	0.355	0.554	0.739	captive bred PCBC	30/11/2006	F
13-219	BH240	0.100	0.355	0.612	0.739	captive bred PCBC		F
13-333	BH74	0.100	0.355	0.548	0.739	captive bred PCBC		F
13-335	BH190	0.100	0.355	0.613	0.739	Bernier Hospital Bay	26/08/2013	F

13-338	BH191	0.100	0.355	0.535	0.739	Bernier Hospital Bay	26/08/2013	F
13-264	0006B36A26	0.200	0.710	0.308	0.740	Dorre Is. White Beach	16/08/1996	F
13-237		0.200	0.710	0.465	0.775	Dorre Island Castle	8/06/2013	?
13-170	0006E229FB	0.100	0.355	0.658	0.809	Bernier Hospital Bay	15/10/2010	F
13-193	BH153	0.100	0.355	0.533	0.809	captive bred PCBC	12/12/2008	M
13-194	BH155	0.100	0.355	0.669	0.809	captive bred PCBC	15/04/2009	F
13-200	BH123	0.100	0.355	0.533	0.809	captive bred PCBC	23/11/2006	M
13-156	0006E21FE0	0.100	0.355	0.593	0.837	captive bred PCBC	26/07/2011	M
13-158	0006E24942	0.100	0.355	0.643	0.837	captive bred PCBC	12/08/2010	F
13-184	0006E2B61F	0.100	0.355	0.578	0.837	captive bred PCBC	15/02/2010	M
13-199		0.100	0.355	0.593	0.837	captive bred PCBC	5/12/2006	F
13-204		0.100	0.355	0.677	0.837	captive bred PCBC	26/07/2006	M
13-273		0.100	0.355	0.618	0.837	Dorre Is. White Beach	9/09/1999	M
13-220	BH249	0.100	0.355	0.652	0.837	captive bred PCBC		F
13-171	0006E24ED7 & 0006E23C58	0.100	0.355	0.569	0.840	Bernier Hospital Bay	19/10/2010	F
13-213		0.100	0.355	0.673	0.840	captive bred PCBC	17/10/2007	F
13-144	Dryandra1	0.100	0.355	0.606	0.843	Return to Dryandra - South Enclosure	22/05/2001	F
13-149	Dryandra6	0.100	0.355	0.606	0.843	Return to Dryandra - South Enclosure	2/02/2001	F
13-173	0006E232F6	0.100	0.355	0.554	0.843	Bernier Hospital Bay	16/08/2011	M
13-162	dam65	0.000	0.000	1.000	1.000	Bernier Hospital Bay	11/08/2010	?
13-166	0006E Chloe	0.000	0.000	1.000	1.000	captive bred PCBC	2/07/2010	F
13-210	ear tag 788	0.000	0.000	1.000	1.000	captive bred PCBC	18/09/2007	F
13-142		0.000	0.000	1.000	1.000	? Dorre Island / ? White Beach	?	?